

Appendix I: Alignment of SEQ ID NO: 7 with the chloramphenicol resistance gene of Borges et al.

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<!--StartFragment-->RESULT 3
AAS00462
ID   AAS00462 standard; DNA; 2003 BP.
XX
AC   AAS00462;
XX
DT   11-JUN-2007 (revised)
DT   16-MAY-2001 (first entry)
XX
DE   Plasmid pLOI2225 useful for chromosomal integration of heterologous DNA.
XX
KW   Plasmid; vector; antibiotic resistance; ethanol; alcohol dehydrogenase;
KW   adhB; pyruvate decarboxylase; pdc; chloramphenicol acetyl transferase;
KW   cat; regulatory element; adhE; chromosomal integration; circular; cyclic;
KW   pLOI2225; pLOI2222; pSG76-C; chloramphenicol resistance;
KW   FRT recombining site; ds.
XX
OS   Synthetic.
XX
FH   Key                Location/Qualifiers
FT   CDS                complement(1045..1704)
FT                       /*tag= a
FT                       /note= "Chloramphenicol-resistance gene"
XX
PN   WO200118222-A1.
XX
PD   15-MAR-2001.
XX
PF   18-AUG-2000; 2000WO-US022700.
XX
PR   07-SEP-1999; 99US-00390479.
XX
PA   (UYFL ) UNIV FLORIDA.
XX
PI   Borges AC, Zaldivar J, Morales FM, Jimenez AM, Ingram LO;
XX
DR   WPI; 2001-235205/24.
DR   PC:NCBI; gi6467484.
XX
PT   Novel nucleic acid construct for integrating heterologous nucleic acid
PT   sequences into genome or chromosome of host cells, has passenger and
PT   marker sequences, in which marker sequence is flanked by recombining
PT   sites.
XX
FS   Claim 28; Page 59-60; 85pp; English.
XX
CC   The present sequence for plasmid pLOI2225 which is constructed from the
CC   plasmids pLOI2222 and pSG76-C is 1 of 7 novel plasmid constructs
CC   (AAS00460-AAS00466) comprising a marker sequence such as an antibiotic
CC   resistance gene, in which the marker sequence is flanked by two FRT
CC   recombining sites. One of these plasmids (pLOI2223) also comprises a
CC   passenger sequence. The passenger sequence can include an ethanologenic
CC   gene such as alcohol dehydrogenase (preferably adhB) or pyruvate
CC   decarboxylase (pdc), another gene such as chloramphenicol acetyl
CC   transferase (cat), a regulatory element such as a promoter or IRES
CC   (internal ribosomal entry site) or a guide sequence such as adhE. All the
CC   plasmids are useful for integrating a nucleic acid construct into the
CC   genome of a cell. Plasmid pLOI2223 is useful for producing ethanol by,
CC   transforming an ethanologenic cell with the plasmid and contacting the
CC   cell with a substrate which can be fermented into ethanol, where
CC   expression of the passenger sequence results in the production of

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CC ethanol. The recombinant ethanologenic host transformed with the plasmid  
 CC has improved properties including increased ability to produce ethanol,  
 CC depolymerisation for a particular substrate and increased tolerance to a  
 CC higher level of ethanol

CC Revised record issued on 11-JUN-2007 : Enhanced with precomputed  
 CC information from BOND.

XX

SQ Sequence 2003 BP; 558 A; 425 C; 436 G; 584 T; 0 U; 0 Other;

Query Match 100.0%; Score 1069; DB 1; Length 2003;  
 Best Local Similarity 100.0%; Pred. No. 0;  
 Matches 1069; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy	1	GCAAAAATTAATAATGAAGTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTG	60
Db	935	GCAAAAATTAATAATGAAGTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTG	994
Qy	61	ACAGTTACCAATGCTTAATCAGTGAGGCACCAATAACTGCCTTAAAAAAATTACGCCCG	120
Db	995	ACAGTTACCAATGCTTAATCAGTGAGGCACCAATAACTGCCTTAAAAAAATTACGCCCG	1054
Qy	121	CCCTGCCACTCATCGCAGTACTGTTGAATTCATTAAAGCATTCTGCCGACATGGAAGCCA	180
Db	1055	CCCTGCCACTCATCGCAGTACTGTTGAATTCATTAAAGCATTCTGCCGACATGGAAGCCA	1114
Qy	181	TCACAGACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTGCGCTTGC	240
Db	1115	TCACAGACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTGCGCTTGC	1174
Qy	241	TAATATTGCCCCATGGTGA AACCGGGGCGAAGAGTTGTCATATTGCCACGCTTTAAA	300
Db	1175	TAATATTGCCCCATGGTGA AACCGGGGCGAAGAGTTGTCATATTGCCACGCTTTAAA	1234
Qy	301	TCAAACTGGTGA AACTCACCAGGGATTGGCTGAGACGAAAAACATATTCTCAATAAAC	360
Db	1235	TCAAACTGGTGA AACTCACCAGGGATTGGCTGAGACGAAAAACATATTCTCAATAAAC	1294
Qy	361	CCTTTAGGGAATAGGCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATGTGT	420
Db	1295	CCTTTAGGGAATAGGCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATGTGT	1354
Qy	421	AGAAACTGCCGGAATCGTCGTGGTATTCACCTCCAGAGCGATGAAAACGTTTCAGTTTC	480
Db	1355	AGAAACTGCCGGAATCGTCGTGGTATTCACCTCCAGAGCGATGAAAACGTTTCAGTTTC	1414
Qy	481	TCATGGAACCGGTGTAACAGGGTGAACACTATCCCATATCACCAGCTCACCCTCTTTC	540
Db	1415	TCATGGAACCGGTGTAACAGGGTGAACACTATCCCATATCACCAGCTCACCCTCTTTC	1474
Qy	541	ATTGCCATACGGAATTTCCGGATGAGCATTATCAGCGGGCAAGAATGTGAATAAAGGCC	600
Db	1475	ATTGCCATACGGAATTTCCGGATGAGCATTATCAGCGGGCAAGAATGTGAATAAAGGCC	1534
Qy	601	GGATAAACTTGTGCTTATTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGA	660
Db	1535	GGATAAACTTGTGCTTATTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGA	1594
Qy	661	ACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGA	720
Db	1595	ACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGA	1654

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Qy      721  TGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTCTCCATTTTAGCTTCC 780
      |||
Db      1655 TGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTCTCCATTTTAGCTTCC 1714

Qy      781  TTAGCTCCTGAAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTTCATTA 840
      |||
Db      1715 TTAGCTCCTGAAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTTCATTA 1774

Qy      841  TGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGGCC 900
      |||
Db      1775 TGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGGCC 1834

Qy      901  CAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTTCTGCGAAGTGATCTTCC 960
      |||
Db      1835 CAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTTCTGCGAAGTGATCTTCC 1894

Qy      961  GTCACAGGTATTTATTCGGCGCAAAGTGCCTCGGGTGATGCTGCCAACTTACTGATTAG 1020
      |||
Db      1895 GTCACAGGTATTTATTCGGCGCAAAGTGCCTCGGGTGATGCTGCCAACTTACTGATTAG 1954

Qy      1021 TGTATGATGGTGTTTTTGAGGTGCTCCAGTGGCTTCTGTTTCTATCAGC 1069
      |||
Db      1955 TGTATGATGGTGTTTTTGAGGTGCTCCAGTGGCTTCTGTTTCTATCAGC 2003

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<!--EndFragment-->